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POTENTIAL ROLE OF $AGNO_3$ IN *IN VITRO* MULTIPLICATION OF POMEGRANATE (*PUNICA GRANATUM* L.) CV. 'BHAGWA'

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ABSTRACT

Silver nitrate induced MS media enhanced multiple shoot regeneration and in vitro growth of Punica granatum (L.) cv 'Bhagwa' an nutritive rich arid fruit crop. Healthy second nodal segments were inoculated on MS medium supplemented with BAP (1.00 mg/l) + NAA (0.25mg/l), BAP (1.00 mg/l) + Kinetin (0.50 mg/l) and BAP (1.00 mg/l) + $AgNO_3$ (1 .00mg/l). The effective and good regeneration capacity of nodal explants was observed in all the combinations of silver nitrate tested when compared to other treatments. Maximum number of multiple shoots (5.50), maximum length of shoot (5.04 cm) and maximum number of leaves (14.08) were found in second nodal segment cultured on MS media supplemented MS B + BAP 1.00 mg/l + $AgNO_3$ 1.00 mg/l. These regenerated shoots were subjected to 3 sub-cultures and they were transferred to rooting medium supplemented with ½ MS B with IBA 1.00 mg/l which recorded maximum root parameters.

KEYWORDS: In Vitro Growth, Micro Propagation, Silver Nitrate, & Punica Granatum

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Original Article

INTRODUCTION

Pomegranate is an economically important species of the world due to its high health benefits and attractive fruits. Every part of the plant has got medicinal importance in one and the other ways. The present scientific name of pomegranate *i.e Punica granatum* L is derived from the name Pomum (apple) granatus (grainy) or seeded apple, which lies in the distinct family Punicaceae, comprised of two species; *P. granatum* and *P. protopunica*. Pomegranate is one of the oldest known fruit trees of the tropics and sub-tropics, cultivated for its delicious edible fruits. It is considered native to Iran. Pomegranate is exploited for nutritional value of its fruit, medicinal properties of different parts of the tree and for ornamental purpose (Parmar and Kaushal, 1982; Naovi *et al*., 1991; Jayesh and Kumar, 2004; Johanningsmeier and Harris, 2011). *In vitro* clonal propagation of pomegranate is advantageous and can be utilized as an alternative to fulfill the demand of good quality and disease free planting material in large scale. However in commercial tissue culture, plant multiplication is carried out through auxiliary shoot proliferation. It is preferred mainly because of its true to type and genetically stable plantlets. Protocols for plant regeneration *in vitro* through stimulation of auxiliary shoot proliferation from nodal stem segments and apical buds or through organogenesis or embryogenesis directly from various explants or callus have been developed for

many important tropical and temperate fruit trees (Hutchinson and Zimmerman, 1987; Litz and Jaiswal, 1991; Grosser, 1994; Zimmerman and Swartz, 1994).

Callus growth, shoot regeneration and somatic embryogenesis can effectively achieved by ethylene in *in vitro.* Thus, by regulating the, the growth and development of some tissue cultures can be controlled to a certain extent by regulating the production or action of ethylene. Some of the observations *viz.*,$AgNO_3$ has been known to inhibit ethylene action (Beyer, 1976) and cobaltous ions are known to inhibit ethylene synthesis (Lau and Yang, 1976), silver ion is capable of specifically blocking the action of exogenously applied ethylene in classical responses such as abscission, senescence and growth retardation led to its application in tissue culture. $AgNO_3$ in culture media greatly improves the regeneration of both dicot and monocot plant tissue cultures (Beyer, 1976; Duncan *et al*. 1985; Davies, 1987; Purnhauser *et al*. 1987; Songstad *et al*. 1988; Chi and Pua, 1989; Veen and Over Beek, 1989; Bais *et al*. 2000; Giridhar *et al*. 2003).

MATERIALS AND METHODS

Source of Plant Material

Healthy explants of *Punica* granatum (L.) were collected from two year old healthy and vigorously growing mother plant of pomegranate cv. 'Bhagwa' (Figure 1) grown at fruit orchard (Main Horticulture Research and Extension Centre), UHS, Bagalkot.

Surface Sterilization

Twigs containing shoot tip as well as 3-4 nodes were taken from the current season shoots of mature tree. The explants were washed thoroughly in running tap water to remove debris.They were further washed 3-4 times with distilled water containing few drops of antiseptic (Tween-20) solution. Further explants washed 5-6 times under running tap water in order to remove the adhered solution completely. Surface sterilization with mercuric chloride ($HgCl_2$) of 0.03 % for 2-3 minutes.

Culture Medium

The explants were inoculated on MS medium containing 30 gm sucrose and 6-7 gm of agar, supplemented with various concentrations of BAP, Kn and AgNO3. The pH of the medium was adjusted to 5.8 before gelling with agar and autoclaved for 20 minutes at 121˚C and 15 lbs pressure**.**

Sub Culturing

The inoculated explants and cultures were transferred on to a fresh multiplication medium 25-30 days after incubation to minimize problems like browning, vitrification and contamination.

Culture Conditions

The culture bottles were incubated in culture room having control over temperature and light. The temperature of culture room was maintained at 25±2°C with light intensity of 16 hours light and 8 hours dark. The photoperiod was controlled using an electronic timer.

RESULTS

Results are furnished in Table 1.

Percent Response for Shoot Initiation

It is an established fact that concentration of cytokinin has a positive formative effect on shoot proliferation. In the present experiment, concentrations of 6-benzylaminopurine (BAP) along with kinetin, 1-naphthalacetic acid (NAA) and silver nitrate ($AgNO_3$) were tried. Significantly the maximum per cent shoot initiation was recorded in treatment T_3 (91.66), which were followed by T_2 (76.00). Significantly the minimum per cent shoot initiation was observed in T_0 (45.00).

Number of Days Taken for Shoot Initiation

The data revealed that day taken for the shoot initiation was found significant. The early shoot initiation observed in treatment T_3 (4.25 days), followed by T_2 (7.42). Delayed shoot initiation was recorded in control *i.e* T_0 (7.83 days).

Number of Shoots per Explant

T_3 had significantly maximum number of shoots per explants (5.50) which was followed by T_2 (2.48). The minimum number of shoots was observed in control *i.e*. T_2 (1.50).

Length of Shoot (Cm)

The effect of growth regulators were found to have significant effect on the mean length of shoot and it was found to be significantly better in the media supplemented with T_3 (5.04 cm) which was followed by T_2 (0.68 cm). The mean length of the shoot was found to be shortest in the media supplemented with T_0 (0.50 cm).

Number of Leaves Per Shoot

Significantly, the highest mean number of leaves per shoot was observed with the media supplemented with T_3 (14.08) which was followed by T_2 (8.75). However, minimum number of leaves was observed in control *i.e*. T_0 (7.63).

The maximum shoot parameters recorded in the presence of $AgNO_3$ which is known to inhibit the ethylene synthesis in the cultures.

DISCUSSIONS

MS B was supplemented with high levels of BAP and $AgNO_3$ resulted better shoot proliferation, which was observed in terms of number of days taken for shoot initiation, number of shoots per explant, length of shoot and number of leaves per shoot. This is mainly due to positive effect of $AgNO_3$ which is strong ethylene inhibitor. Here by this study concludes the role of $AgNO_3$ in *in vitro* shoot regeneration and proliferation in pomegranate.

Table 1: Effect of Different Growth Regulator on Shoot Growth and Proliferation in Pomegranate cv. 'Bhagwa'

Treatments	Media	Shoot Initiation (%)	Number of Days for Shoot Initiation	Number of Shoots / Explant	Length Of Shoot (Cm)	Number Leaves / Shoot
T_0 (control)	MS B	45.00 (42.14)*	7.83	1.50	0.50	7.63
T_1	MS B + BAP 1.00 mg/l + NAA 0.25 mg/l	50.00 (45.00)	7.75	1.62	0.57	7.83
T_2	MS B + BAP 1.00 mg/l + Kinetin 0.50 mg/l	76.00 (60.68)	7.42	2.48	0.68	8.75
T_3	MS B + BAP 1.00 mg/l + $AgNO_3$ 1.00 mg/l	91.66 (73.40)	4.25	5.50	5.04	14.08
	SEm±	0.45	0.04	0.01	0.02	0.06
	CD 1%	1.79	0.16	0.03	0.08	0.23

The values given in parenthesis are arc sine transformed values

Figure 1: Mother Plant

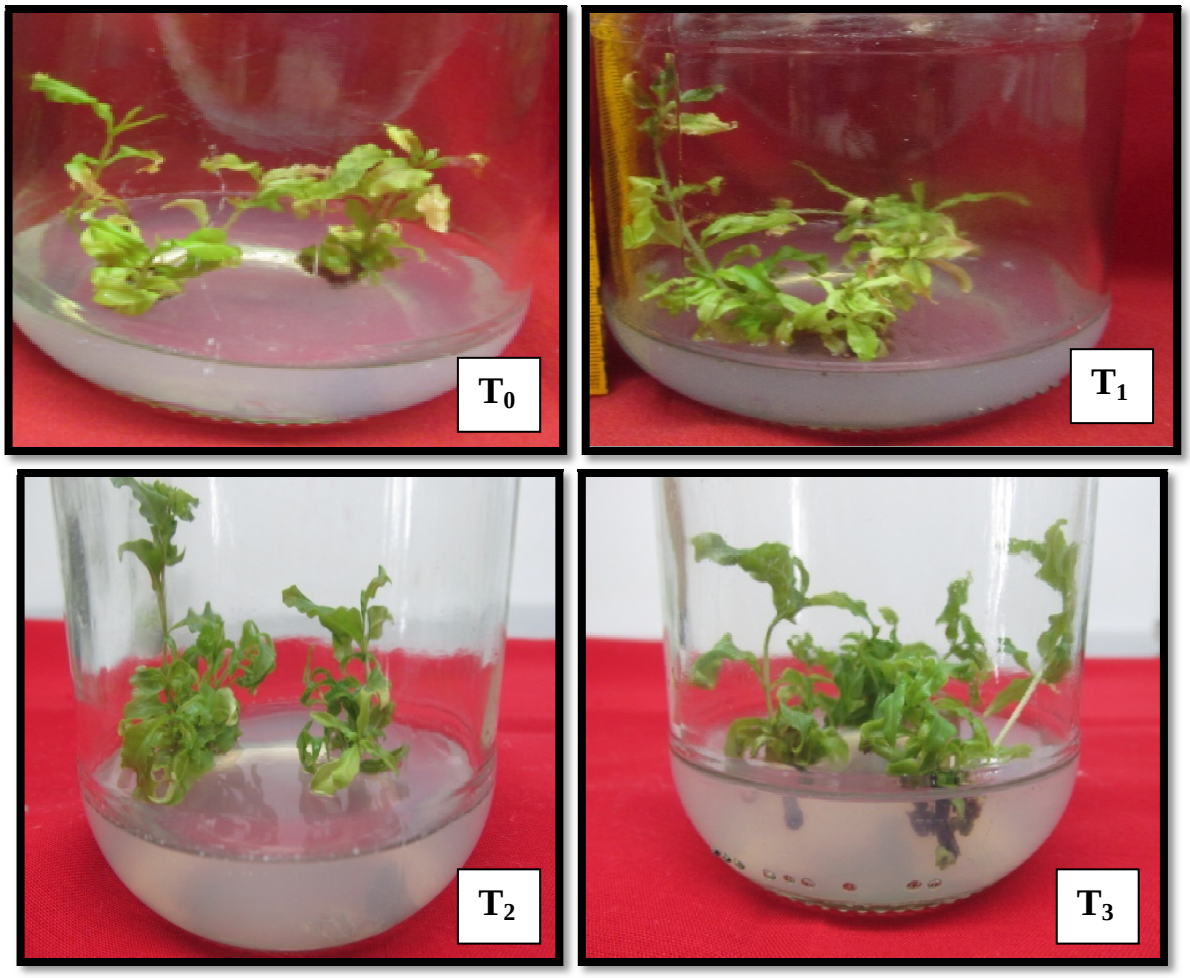

Figure 2: In Vitro Shoot Proliferation and Growth of Pomegranate Cv. 'Bhagwa'

T$_0$: MS B	**T$_2$:**MS B + BAP 1.00 mg/l +Kinetin 0.50 mg/l
T$_1$: MS B + BAP 1.00 mg/l +NAA 0.25 mg/l	**T$_3$** : MS B + BAP 1.00 mg/l +$AgNO_3$ 1.00 mg/l

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